

### **REMARKS**

Applicants respectfully request entry of this Amendment and reconsideration of the outstanding objections and rejections of the claims in the above-identified application.

Applicants have canceled claim 52 without prejudice or disclaimer. Applicants reserve the right to pursue the subject matter of this claim in one or more continuation application.

Applicants have amended claims 43- 45, 46 and 48. The amended claims are supported throughout the specification including at page 6, line 3-21 and page 1 lines 16-18. Applicants submit that the amended claims do not represent new matter.

Applicants have added new claims 53-62. Applicants submit the new claims are supported throughout the specification including at page 5 lines 1-32. Applicants submit that the new claims do not represent new matter.

Applicants have amended the specification in order to insert symbols that were inadvertently deleted in the last amendment. Applicants submit that the amendments do not represent new matter.

### **Interview**

Applicants thank Examiner Wax for the interview conducted on May 24, 2004. In the interview , we discussed the 112, first paragraph enablement and written description rejection of the claims. Amendments to the claims to address the rejections were proposed.

### **35 U.S.C. § 112, First Paragraph, Enablement**

The Examiner has rejected claims 43-52 under 35 U.S.C. § 112, first paragraph. The Examiner contends that the claims are directed to any protein of animal origin that will reversibly bind oxygen when combined with a plant porphyrin and that the quantity of experimentation would be large. The Examiner also contends that claims 45-49 are directed to any variant that will reversibly bind oxygen and that the specification does not provide adequate guidance as to the which mutations can be made. Applicants respectfully traverse the rejection.

There are many factors to be considered in an analysis of enablement, including breadth

of the claims, nature of the invention, the state of the prior art, the level of ordinary skill, level of predictability in the art, the amount of direction provided by the inventor and the existence of working examples, and the quantity of experimentation. MPEP 2164.01(a) citing *In Re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). Only a reasonable correlation between the claims and the scope of enablement is required.

Applicants claims are directed to a recombinant protein comprising a porphyrin nucleus of plant origin and a protein component comprising at least one polypeptide chain selected from the group consisting of hemoglobin, myoglobin, cytochromes, peroxidases and catalases. Applicants have also claimed a recombinant protein comprising a variant of the  $\alpha$  and  $\beta$  chain, wherein the variant of the  $\alpha$  chain has at least 90% homology with an  $\alpha$  chain having an amino acid sequence of SEQ ID NO:31 and the variant of the  $\beta$  chain has at least 90% homology with a  $\beta$  chain having an amino acid sequence of SEQ ID NO:33, and the hemin protein is capable of reversibly binding oxygen. Applicants have also claimed a recombinant hemin protein that comprises at least one  $\alpha$  and/or  $\beta$ -globin polypeptide chain, or variants of said polypeptide chain, wherein the variant of the  $\alpha$  chain has a heme binding domain and the variant of the  $\beta$  chain has a heme binding domain and the hemin protein is capable of binding oxygen reversibly.

Applicants submit that the specification as filed provides sufficient information for how to make and use the claimed invention without undue experimentation. Applicants' claims are now directed to heme binding proteins selected from the group consisting of hemoglobin, myoglobin, cytochromes, peroxidases and catalases. Applicants have described the structure of and shown that the  $\alpha$  and  $\beta$  globin chains can be expressed in plant cells. The heme group is known to be associated with the ability of the protein to reversibly bind oxygen. A working example shows that the  $\alpha$  and  $\beta$  chains can associate with a plant porphyrin and with each other. It was surprising that a molecule as complex as a tetramer could assemble properly and bind a plant porphyrin in plant cells.

The structures of  $\alpha$  and  $\beta$  globin are well known in the art as well as the heme binding domain of each chain. (see for example, Craik et al. PNAS77:1384 (1980))The heme binding site of myoglobin is described in the chapter on hemoglobin from Biochemistry (ed. Stryer). The crystal structures of these protein are also known. The structure of the heme binding domains of the other heme proteins is also known to those of skill in the art. For example, Matsunaga et al

and Soucek et al describe the heme binding region of cytochrome 450 proteins. (copies enclosed) Plants are known to make cytochromes as well. Cytochrome P450 also has some similar structural features to globins (Holm et al., FEBS 315:301-306 (1993)). When the sequences of plant catalases are compared to bovine catalase, conservation of amino acid sequence is seen at the region of the heme binding site. (Ni et al., BBA 1049:219-222 (1990)).

Applicants submit that the claimed proteins share the structural feature of binding to heme and that the structure of the heme binding domain of these proteins is known to those of skill in the art. Applicants submit that they have provided an exemplary structure and the structure/ function relationship of heme binding proteins was known to those of skill in the art. Applicants submit that if a complex molecule like hemoglobin can be produced and properly assembled in plants, then it is reasonable to expect that molecules that have a single chain or a single heme binding site could also be properly assembled in plants. Therefore, Applicants submit they have provided adequate enablement for the claimed invention and respectfully request that the examiner withdraw the rejection on this basis.

The Examiner also contends that the Applicants have not described variants of the  $\alpha$  and  $\beta$  chain. Applicants disagree. Variants are described in the specification at pages 6 - 9. Specific embodiments of variant are described at pages 8 and 9. Moreover, many variants of both the  $\alpha$  and  $\beta$  chain as well as their functional characteristics were known to those of skill in the art at the time of filing of the application. More than 50 such variants are described in Hemoglobin 19:39-124 (1995) (copy enclosed) Therefore, Applicants respectfully request withdrawal of the rejection on this basis.

**35 U.S.C. § 112, First Paragraph, Written Description**

The Examiner has rejected claims 43-52 under 35 U.S.C. § 112, first paragraph. The Examiner contends that the claims are directed to any protein of animal origin that will reversibly bind oxygen when combined with a plant porphyrin. The Examiner also contends that claims 45-49 are directed to any variant that will reversibly bind oxygen and that the specification does not provide disclosure of structure of the variants. Applicants respectfully traverse the rejection.

The written description requirement requires that Applicants' specification must convey

with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. Vas-Cath Inc. v. Mahurkar, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991). As noted in the Guidelines for Examination of Patent Applications Under 35 U.S.C. § 112, ¶1, "Written Description" Requirement ("the guidelines"), there is a "strong presumption" that an adequate written description of the claimed invention is present when the application is filed, 66(4) Fed Reg. 1099, 1105 (2001); see also, In re Wertheim, 191 USPQ 90,97 (CCPA 1976). Compliance with the written description requirement does not require an applicant to describe exactly the subject matter claimed; rather, the description must clearly allow a person of ordinary skill in the art to recognize that he or she invented what is claimed. Vas-Cath Inc. v. Mahurkar, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991). The test is whether the originally filed specification reasonably conveys to a person having ordinary skill in the art that applicant had possession of the subject matter later claimed. In re Kaslow, 217 USPQ 1089 (Fed. Cir. 1991). The specification does not need to describe that which is known to those of skill in the art. Moreover, in order to have possession of members of a claimed genus, the specification need not describe all of the species that the genus encompasses. Amgen Inc. v. Chugai Pharmaceutical Co., 18 USPQ2d 1016, 1027 (Fed. Cir. 1991).

Applicants claims are directed to a recombinant protein comprising a porphyrin nucleus of plant origin and a protein component comprising at least one polypeptide chain selected from the group consisting of hemoglobin, myoglobin, cytochromes, peroxidases and catalases. Applicants have also claimed a recombinant protein comprising a variant of the  $\alpha$  and  $\beta$  chain, wherein the variant of the  $\alpha$  chain has at least 90% homology with an  $\alpha$  chain having an amino acid sequence of SEQ ID NO:31 and the variant of the  $\beta$  chain has at least 90% homology with a  $\beta$  chain having an amino acid sequence of SEQ ID NO:33, and the hemin protein is capable of reversibly binding oxygen. Applicants have also claimed a recombinant hemin protein that comprises at least one  $\alpha$  and/or  $\beta$ -globin polypeptide chain, or variants of said polypeptide chain, wherein the variant of the  $\alpha$  chain has a heme binding domain and the variant of the  $\beta$  chain has a heme binding domain and the hemin protein is capable of binding oxygen reversibly.

Applicants submit that the specification as filed provides adequate written description for the claimed invention. Applicants have described the structure of and shown that the  $\alpha$  and  $\beta$

globin chains can be expressed in plant cells. The heme group is known to be associated with the ability of the protein to reversibly bind oxygen. Once expressed, the  $\alpha$  and  $\beta$  chains can associate with a plant porphyrin and with each other. It was surprising that a molecule as complex as a tetramer could assemble properly and bind a plant porphyrin in plant cells. The structures of  $\alpha$  and  $\beta$  globin are well known in the art as well as the heme binding domain of each chain. The heme binding site of myoglobin is described in the chapter on hemoglobin from Biochemistry (ed. Stryer).

The structure of the heme binding domains of the other heme proteins is also known to those of skill in the art. For example, Matsunaga et al and Soucek et al describe the heme binding region of cytochrome p450 proteins. (copies enclosed) Cytochrome P450 also has some similar structural features to globins (Holm et al., FEBS 315:301-306 (1993). When the sequences of plant catalases are compared to bovine catalase, conservation of amino acid sequence is seen at the region of the heme binding site. (Ni et al., BBA 1049:219-222 (1990)).

Applicants submit that the claimed proteins share the structural feature of binding to heme and that the structure of the heme binding domain of these proteins is known to those of skill in the art. Applicants submit that they have provided an exemplary structure and the structure/ function relationship of heme binding proteins was known to those of skill in the art. Therefore, Applicants submit they have provided adequate written description for the claimed invention and respectfully request that the examiner withdraw the rejection on this basis.

The Examiner also contends that the Applicants have not described variants of the  $\alpha$  and  $\beta$  chain. Applicants disagree. Variants are described in the specification at pages 6 - 9. Specific embodiments of variants are described at pages 8 and 9. Moreover, many variants of both the  $\alpha$  and  $\beta$  chain as well as their functional characteristics were known to those of skill in the art at the time of filing of the application. (See Hemoglobin 19:39-124 (1995) copy enclosed) Therefore, Applicants respectfully request withdrawal of the rejection on this basis.

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Reply to Office Action of December 31, 2003

**SUMMARY**

Applicants submit that all pending claims are in condition for allowance and notification to that effect is earnestly solicited. The Examiner is invited to contact Applicants' representative if prosecution may be assisted thereby.

Respectfully submitted,

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